Final Scientific Report

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BARD Project Number: IS-3886-06

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Project Title: Molecular Analyses of Somaclonal Variation in Date Palm and Banana for Early Identification and Control of Off-types Generation

Investigators	**Institutions**
Principal Investigator (PI):	
Yuval Cohen	ARO, The Volcani center
Co-Principal Investigator (Co-PI):	
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Collaborating Investigators:	
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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Abbreviations commonly used in the report, in alphabetical order**:**

MS-AFLP – Methylation Sensitive AFLP; TC - Tissue culture

Budget: IS: $155,000 US: $129,000 Total: $284,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	0	1	0	1
Submitted, in review, in preparation			2	2
Invited review papers				
Book chapters			1	1
Books			0	
Master theses			2	
Ph.D. theses				
Abstracts			6	6
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Dr. Sekar Makesh (India)
Dr. Shirly Gazit (Israel)
Dr. Hewan Demisse Degu (Ethiopia)

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	0	0	0	0
Longer Visits (Sabbaticals)	0	0	0	0

Description Cooperation:
The cooperation was mainly through electronic communications. The sharing of the plant material was essential for the project to proceed and the wealth of data on the subset of trees has moved forward our understanding of the problem. The experience with this first collaboration is expected to lead to further joint projects based on both partners' interests in the response of plants to various stress environments.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted	0	0	0	0
Issued (allowed)	0	0	0	0
Licensed	0	0	0	0

Abstract

Date palm (*Phoenix dactylifera* L.) is the major fruit tree grown in arid areas in the Middle East and North Africa. In the last century, dates were introduced to new regions including the USA. Date palms are traditionally propagated through offshoots. Expansion of modern date palm groves led to the development of Tissue Culture propagation methods that generate a large number of homogenous plants, have no seasonal effect on plant source and provide tools to fight the expansion of date pests and diseases. The disadvantage of this procedure is the occurrence of off-type trees which differ from the original cultivar. In the present project we focused on two of the most common date palm off-types: (1) trees with reduced fruit setting, in which most of the flowers turn into three-carpel parthenocarpic fruits. In a severe form, multi-carpel flowers and fruitlets (with up to six or eight carpels instead of the normal three-carpel flowers) are also formed. (2) dwarf trees, having fewer and shorter leaves, very short trunk and are not bearing fruits at their expected age, compared to the normal trees. Similar off-types occur in other crop species propagated by tissue culture, like banana (mainly dwarf plants) or oil palm (with a common 'Mantled' phenotype with reduced fruit setting and occurrence of supernumerary carpels). Some off-types can only be detected several years after planting in the fields. Therefore, efficient methods for prevention of the generation of off-types, as well as methods for their detection and early removal, are required for date palms, as well as for other tissue culture propagated crops.

This research is aimed at the understanding of the mechanisms by which off-types are generated, and developing markers for their early identification. Several molecular and genomic approaches were applied. Using Methylation Sensitive AFLP and bisulfite sequencing, we detected changes in DNA methylation patterns occurring in off-types. We isolated and compared the sequence and expression of candidate genes, genes related to vegetative growth and dwarfism and genes related to flower development. While no sequence variation were detected, changes in gene expression, associated with the severity of the "fruit set" phenotype were detected in two genes - PdDEF (Ortholog of rice SPW1, and AP3 B type MADS box gene), and PdDIF (a defensin gene, highly homologous to the oil palm gene EGAD). We applied transcriptomic analyses, using high throughput sequencing, to identify genes differentially expressed in the "palm heart" (the apical meristem and the region of embryonic leaves) of dwarf vs. normal trees. Among the differentially expressed genes we identified genes related to hormonal biosynthesis, perception and regulation, genes related to cell expansion, and genes related to DNA methylation. Using Representation Difference Analyses, we detected changes in the genomes of off-type trees, mainly chloroplast-derived sequences that were incorporated in the nuclear genome and sequences of transposable elements. Sequences previously identified as differing between normal and off-type trees of oil palms or banana, successfully identified variation among date palm off-types, suggesting that these represent highly labile regions of monocot genomes.

The data indicate that the date palm genome, similarly to genomes of other monocot crops as oil palm and banana, is quite unstable when cells pass through a cycle of tissue culture and regeneration. Changes in DNA sequences, translocation of DNA fragments and alteration of methylation patterns occur. Consequently, patterns of gene expression are changed, resulting in abnormal phenotypes. The data can be useful for future development of tools for early identification of off-type as well as for better understanding the phenomenon of somaclonal variation during propagation *in vitro*.

Achievements

In the present project we focused on molecular characterization of the most common date palm off-types: (1) trees (cv.'Barhee') with reduced fruit setting, in which most of the flowers turn into three-carpel parthenocarpic fruits. In a severe form, multi-carpel flowers and fruitlets (with up to six or eight carpels instead of the normal three-carpel flowers) are also formed. (2) dwarf trees (cv. 'Medjool'), having fewer and shorter leaves, very short trunk and do not bear fruits at the expected age. Various molecular approaches have been used to elucidate the events resulting in the formation of the off-types and in order to develop markers for their early detection.

DNA-methylation patterns in normal and off-type trees: methylation patterns are cultivar specific and are relatively conserved among off-shoots propagated trees of the same cultivar. Several alterations in the DNA methylation patterns were detected among off-types by Methylation Sensitive – AFLP. Nine variations distinguishing between normal and off-type trees were identified, and two of them were confirmed by bisulfite sequencing. These results support the hypothesis that changes in DNA methylation patterns and/or sequence variation, occurring during the tissue culture propagation, are associated with off-type phenotypes identified in the orchard, years after planting.

Isolation and characterization of candidate genes related to flower development and dwarfism: Phenotypes similar to ‘dwarfism’ and 'low fruit set' occur in other plant species like oil-palm and banana. We identified conserved fragments in 14 genes related to plant stature and in 10 genes related to flower development. A diffensin gene, (suggested to be involved in the formation of the oil palm 'Mantled' off-type phenotype, (Tregear et al., J. Exp. Bot. 53, 1387, 2002)), was also identified. Although sequence variations were identified, no association with a specific phenotype could be detected. The expression patterns of two genes, PdDEF (Ortholog of rice SPW1, and AP3 B type MADS box gene), and PdDIF (a defensin gene, highly homologous to the oil palm gene EGAD), is reduced in the flowers of 'Barhee' off-types with the severe phenotype of ‘low fruit set' (producing extra supernumerary carpels). The reduced expression of the PdDEF gene may be associated with the formation of the extra carpels. The alleviation of the 'Low fruit set' phenotype during several years in the orchards, and the change in the expression of the PdDEF and

PdDIF genes suggest a possible epigenetic control of this gene in the formation of the phenotype. We sequenced the entire coding sequence and most of the genomic sequence of PdDEF from normal and off-type 'Barhee' trees but no differences in sequence could be detected. Analysis of the methylation patterns of 1350 bps fragment at the 5' of the gene did not detect high levels of methylation, suggesting that the PdDEF gene is not directly controlled by DNA methylation. However, control of PdDEF by other epigenetic mechanism, or DNA methylation of upstream genes, are not excluded.

Transcriptome analysis of dwarf vs. normal trees: Two approaches were applied to characterize the transcriptomes of the "heart" of date palms to identify genes that are differentially expressed in dwarf 'Medjool' trees: 1. Heterologous gene chip hybridization to rice Affymetrix chips and 2. High throughput sequencing by the Solexa technology. This technology was applied by Fasteris, Swiss and included both Digital Gene Expression and Paired-End mRNA-Seq (which was applied after the preliminary date palm genome draft became available). More than 1700 genes were found to have differential expression under stringent filtering conditions. Q-RT-PCR analyses of selected genes confirmed the differences in gene expression between the normal and the dwarf trees. Many of the differentially expressed genes are related to hormonal biosynthesis, perception and regulation, cell expansion, and DNA methylation. Further analysis of these genes may enable better understanding of the molecular events occurring in dwarf off-types of dates and other species.

Detection of genomic DNA changes, using Representation Difference Analysis: Following separate sets of subtractions of normal vs. off-type (for either 'dwarf' and 'fruit set' phenotypes) DNAs, a total of 1437 contigs were identified in the “subtraction products”. The two most frequent hits were to chloroplast sequences and to parts of retrotransposons. The chloroplast-related sequences are likely to have arisen in chloroplast-derived sequences present in the nuclear genome. The most commonly occurring sequence was a part of the 16S ribosomal RNA region from the chloroplast genome. Since the genes associated with transposable elements were not intact elements, it is most likely that the activation of two element systems had occurred.

Screening of Normal and Off-type plants with heterologous primers derived from Banana and Oil Palm: Two sets of DNA sequences, derived from a comparison of normal and dwarf off-type banana plants and from a comparison of normal and

'Mantled' flower tissue culture derived oil palm plants, were used to amplify differential bands from various date palm trees. Although differences were found, they were not robust enough to be used as practical markers for identification of off-types. These results suggest that there is a very labile set of regions within the genome that vary rapidly in tissue culture. The same sets of regions are present and labile in various monocot genomes (namely, banana, oil palm and date palm). The regions identified as different in the comparison between date offshoot and tissue culture-derived plants, were also variable when 19 various date palm cultivars were compared. These polymorphisms enabled to unambiguously identify each of the date palm cultivars. However, further analysis revealed that the variation among these date palm varieties lies mainly near or at the original primer sites and that the internal sequences are conserved.

Sequence variation, unstable gene fragments, DNA methylation and consequently gene expression were found to be associated with the off-type phenotypes. The data indicate that the date palm genome is quite unstable when cells pass through a cycle of tissue culture and regeneration. The number and nature of the difference alterations has made the identification of the specific variation associated with a known somaclonal phenotype difficult to ascertain. Further research will explore the nature and position of the genomic variants and identify those common to somaclonal variants. Future studies will also focus on identification of the causal changes that result in the formation of specific off-types, for better understanding of the somaclonal phenomenon.

List of publications

Publications in pear review journals

Johnson C, Cullis TA, Cullis MA, Cullis CA: DNA markers for variety identification in date palm (*Phoenix dactylifera* L.). ***J. Hort. Sci. Biotech***. 2009, 84:591-594.

Book chapters

Cohen Y. Molecular approaches for early detection of somaclonal variation in date palms. In: Date Palm Biotechnology. (Jain, S.M., Al-Khayri, J., Johnson, D.V., Eds) Springer; 2011.